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IN SILICO EXPRESSION ANALYSIS OF THE QTL REGION ASSOCIATED WITH ROOT TRAITS IMPARTING DROUGHT TOLERANCE IN RICE

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ABSTRACT

Rice (Oryza sativa L.) is the only cereal produced for solely human consumption and is also a cereal of high social and economic value. Water deficit is the main constraint that affects the yield in rice production. Rain fed rice have different drought types and their characterization for the region concerned is essential for identifying drought resistant traits. Therefore, rice varieties with characteristic of efficient use of water are needed to be developed. To understand gene discovery, genetic engineering of rice and mechanism of drought tolerance, fundamental knowledge of plant responses to a biotic stresses at the genomic level is essential. BLAST search was used to obtain the physical position of each flanking QTL interval marker in RGP (Rice Genome Project). All the BAC/PAC clones underlying QTL marker interval were procured and then the genes on sequences were obtained. The expression analysis of the genes of the identified BAC/PAC sequences were performed using MPSS technique. A total of 2568 genes were identified in the putative region of QTLs under study. Genes identified in the putative region of chromosome 1 were 224, chromosome 2 were 388, chromosome 3 were 19, chromosome 4 were 866, chromosome 9 were 1071. Out of these genes, the genes classified as Kinase-like(1), Receptor kinase (1), Transcription factor(8), Aquaporin(1), Protein kinase(11) were chosen for further analysis as it is reported that they play a significant role in drought tolerance.

KEYWORDS: MPSS, QTL, BAC/PAC, TPM, BLAST & RGP

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Original Article

1. INTRODUCTION

Rice is the staple food for half of the world's population and requires more water than all the other major cereals (To orchi *et al.*, 2003). For improved drought tolerance in the rain fed lowland ecosystem, as compared with the experience of upland rice a deep and thick root system is advantageous (O'Toole, 1982; and Fukai and Cooper, 1995). A positive effect on subsequent plant growth and root system development during progressive water stress under anaerobic well-watered conditions were reported by Azhiri-Sigari *et al.*, 2000 and Kobata, 1994. Improved water extraction was observed as a result of greater root elongation to depth. Wade *et al.*, 1999 reported that in spite of having fewer roots in deeper layers, rain fed lowland rice can extract water from below a15-cm soil depth. Ekanayake *et al.* 1985; O'Toole and Chang 1979 Yoshida and Hasegawa, 1982reported that there is an association between root characteristics and drought avoidance in rice.

Root morphological QTLs were mapped and related these to QTLs for field drought resistance by Champoux *et al.* (1995). A QTL qSOR1was identified by Yusaku *et al.* (2011) for soil surface rooting. Studies

suggest that the application of markers to the study of drought stress can greatly enhance our understanding of physiologically complex traits. Rice breeders will benefit from the markers identified.

2. MATERIALS AND METHODS

Computational Genomics for Prediction of Gene Expression

In Silico Expression Profiling of Putative Drought-Tolerant Genes Underlying the Putative QTL Regions

The URL http://www.mpss.udel.edu/rice that contains rice MPSS database and includes comprehensive set of libraries used for the study. An exact digital representation of copies of the transcript in a tissue which indicates expression level of the corresponding gene quantitatively is TPM (Transcript per Million), 17 and 20 bases signatures is the final output of MPSS (Brenner *et al.* 2000; Meyers *et al.* 2004). Chen and Rattray, 2006 proposed that study of the relative expression level of different genes within a sample and relative concentration of almost all mRNA molecules within a cell population can be done using TPM value. BLAST search was used to obtain the physical position of each flanking QTL interval marker in RGP (Rice Genome Project).

All the BAC/PAC clones underlying QTL marker interval were procured and then genes on sequences were obtained. Based on publicly available information the genes on sequences encompassing QTL intervals were categorized for drought tolerance in general and root traits in particular (http://rice.plantbiology.msu.edu). To refine the putative region identified in the present study 2568 drought-tolerant genes associated with root traits were downloaded. The putative QTL regions are found to harbor key drought tolerant genes namely transcription factor, kinase like, protein kinase, receptor kinase and aqua porins.

A total of 36 genes belonging to Kinase-like, Receptor kinase, Transcription factor, Aquaporin and Protein kinase categories were identified on the various putative QTL on which the analysis was conducted. On chr#1, 7 Transcription factor genes were found. On chr#2, 1 Transcription factor and 2 protein kinase genes were identified. Chr#4 was found to have 1 aquaporin and 4 protein kinase genes. The maximum number of genes were identified on chr# 9, 1 each of kinase like and receptor kinase, 8 of transcription factor and 11 of protein kinase. Out of these genes, the genes classified as Kinase-like, Receptor kinase, Transcription factor, Aquaporin, Protein kinase were chosen for further analysis as it is reported that they play a significant role in drought tolerance.

Table 1: Frequency Distribution of Expressed Genes in the Putative QTL Region

	Kinase-like	Receptor kinase	Transcription factor	Aquaporin	Protein Kinase
Chr#1			7		
Chr# 2			1		2
Chr#4				1	4
Chr#9	1	1	8		11

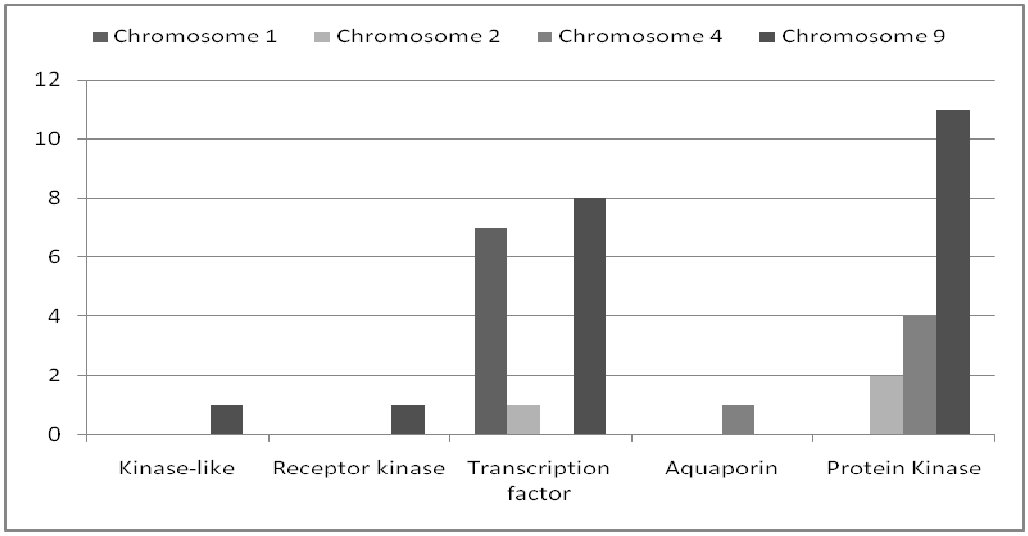

Figure 1: Chromosome-Wise Frequency Distribution of Putative Expressed Genes Significantly Contributing to Drought Tolerance.

Table 2: Class Wise Distribution of MPSS Signature Tags

Class of MPSS tags	Position of MPSS tags	Number of Signature tags				
		Chr1	Chr2	Chr4	Chr9	Total
1	Within exon same strand	31	23	24	170	248
2	Within 500bp potential 3_UTR	11	7	12	29	59
3	Antisense to exon	31	23	24	123	201
4	Unannotated	0	0	0	1	1
5	Within intron sense strand	35	34	10	85	164
6	Within intron antisense strand	35	33	10	86	164
7	Spans an exon/intron splice site	0	3	0	0	3
Total		143	123	80	494	840

In the putative QTL region, a total of 840 MPSS tags were found to be located. 248 the maximum number belonged to class-1 (Within ex on same strand) and rest belonged to 2(59), 3(201), 4(1), 5(164), 6(164), 7(3) classes of MPS signature tags. Based on the frequency of TPM out of 52 loci 4 loci were containing MPSS tag having TPM value greater than 500. Class wise distribution is given in Table 2.

A total of 143 MPSS tags(17bp) were identified in the putative drought tolerant loci in the QTL region of chromosome 1. Out of the total 143 tags maximum tags (35) belonged to class 5 and followed by class 1 and 3 (31), and then class 2 with11 tags, class 4 had nil tags. Based on the frequency of TPM out of 52 loci 4 loci were containing MPSS tag having TPM value greater than 500. A total of 123 MPSS tags (17bp) were identified in the putative drought tolerant loci in the QTL region of chromosome 2. Maximum tags belonged to class 5(34), followed by class 6(33), followed by class 1 and 3 both with equal number of tags (23), class 2 had 7 tags whereas tags corresponding to class 4 were nil. A total

of 80 MPSS tags (17bp) were identified in the putative drought-tolerant loci in the QTL region of chromosome 4. Maximum tags belonged to class 1 and 3(24), followed by class 2 with 12 tags, class 5 and 6 had 10 tags whereas class 7 had no tags. A total of 494 MPSS tags (17bp) were identified in the putative drought tolerant loci in the QTL region of chromosome 9. Class 1 had maximum number of tags (170), followed by class 3 with 123 tags, class 6 and 5 had 86 and 85 tags respectively and class2 had 29 tags. No tags were reported in class 7.

High TPM tags corresponding to 4 putative drought loci with MPSS tag GATCTTCTGGGCGGGGC corresponding to **LOC_Os04g16450** showed the highest TPM value 4539 in FRO, 3 209 in FRR, 1431 in FME, 1244 in 9RO and 785 in 9ME. High TPM tags corresponding to 12 putative drought loci with MPSS tag GATCAAGAACTACTGGA corresponding to **LOC_Os09g36730** showed the highest TPM value 1571 in FRR and 1492 in FRO, TPM 771 in 9ME and 756 in FLA. High TPM tags corresponding to 19 putative drought loci corresponding to **LOC_Os09g20350** with high TPM value 590 in FLB. High TPM tags corresponding to 41 putative drought loci with MPSS tag GATCCAAAAGTGGCGGC corresponding to LOC_Os02g32610showed the highest TPM value in 9LC. The details of the high TPM value in the express tissue libraries is given in Table 3.

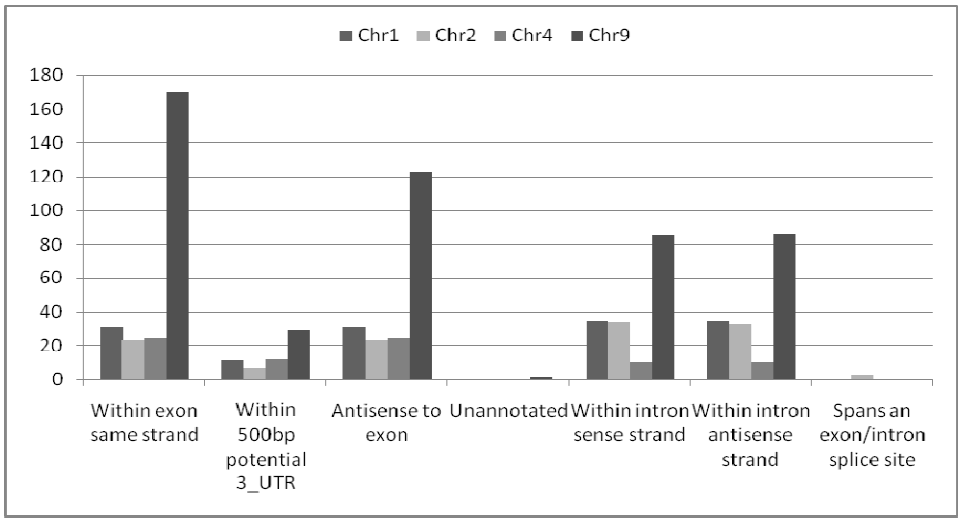

Figure 2: Frequency Distribution of MPSS Signature Tags.

Table 3: Expression of Signature Tags in all 14 mRNA Libraries

Type of gene	Chromosome	Accession No.	Locus ID	9LA	9LB	9LC	9LD	9ME	9RO	9RR	FLA	FLB	FLC	FLD	FME	FRO	FRR
Transcription factor	**chr 9**	AP006 174	LOC_Os09g 36730	217	207	77	198	771	283	505	756	210	122	146	487	1574	1652
Transcription factor	**chr9**	AP005 525	LOC_Os09g 20350	492	263	253	268	92	439	165	265	590	292	319	134	105	107
Aquaporin	**chr 4**	AL731 636	LOC_Os04g 16450	0	60	0	0	785	1244	195	0	28	0	0	1431	4539	3209
Protein kinase	**chr2**	AP004 777	LOC_Os02g 32610	185	252	779	507	292	39	81	48	340	328	475	233	62	136

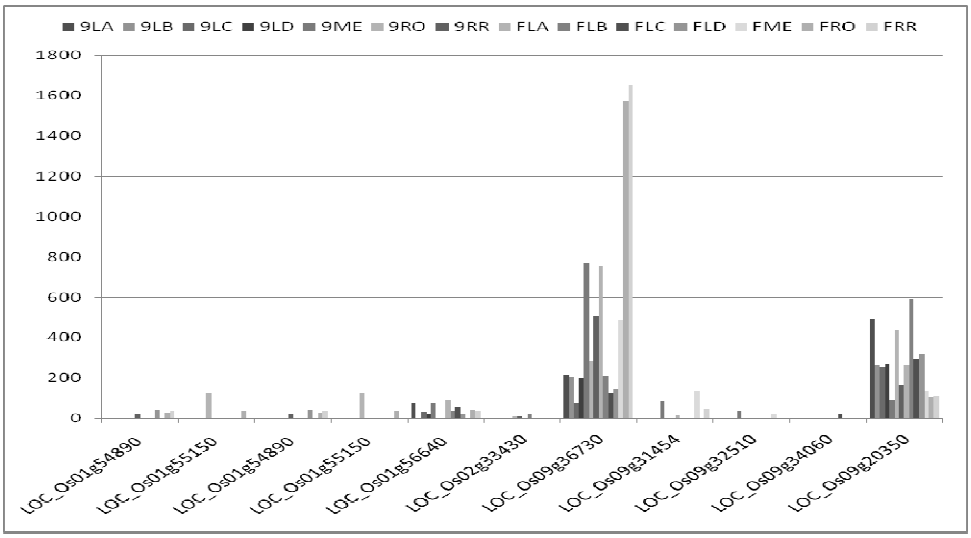

Figure 3: Expression Pattern of Genes Identified in the QTL Regions based on MPSS Signature Abundance in different Tissue Libraries.

4. CONCLUSIONS

Genes related to stress responses can be identified with the help of bioinformatics tools and expression analysis. A total of 2568 genes were identified in the putative region of QTLs under study. Genes identified in the putative region of chromosome 1 were 224, chromosome 2 were 388, chromosome 3 were 19, chromosome 4 were 866, chromosome 9 was1071. Among the 840 MPSS tags (17 bp) which were found to be located in the putative QTL region. Maximum number of signature tags 248 belonged to class-1(Within ex on same strand) and rest belonged to 2(59), 3(201), 4(1), 5(164), 6(164), 7(3) classes of MPSS signature tags. Based on the frequency of TPM out of 52 loci 4 loci were containing MPSS tag having TPM value greater than 500. High TPM tags corresponding to 4 putative drought loci with MPSS tag GATCTTCTGGGCGGGGC corresponding to **LOC_Os04g16450** showed the highest TPM value 4539 in FRO, 3209 in FRR, 1431 in FME, 1244 in 9RO and 785 in 9ME. High TPM tags corresponding to 12 putative drought loci with MPSS tag GATCAAGAACTACTGGA corresponding to **LOC_Os09g36730** showed the highest TPM value 1571 in FRR and 1492 in FRO, TPM 771 in 9ME and 756 in FLA. High TPM tags corresponding to 19 putative drought loci corresponding to **LOC_Os09g20350** with high TPM value 590 in FLB. High TPM tags corresponding to 41 putative drought loci with MPSS tag GATCCAAAAGTGGCGGC corresponding to LOC_Os02g32610showed the highest TPM value in 9LC. These MPSS tags may serve as a source for identification, analysis and functional characterization of genes controlling a biotic stress. The molecular markers generated in the study can directly be used for valid a ting in the breeding population for Molecular Assisted Selection of root parted and drought-related traits in general.

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